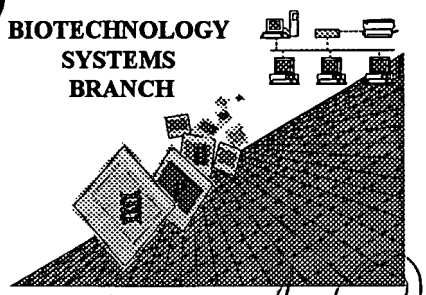
McHarry

Feb 6 99

BIOTECHNOLOGY SYSTEMS BRANCH



RAW SEQUENCE LISTING ERROR REPORT

#8

The Biotechnology Systems Branch of the Scientific and Technical Information Center (STIC) detected errors when processing the following CRF diskette:

JRH 6-5-98

Application Serial Number: 08/822,963

Art Unit / Team No. : 1635

Date Processed by STIC: 5/5/98

THE ATTACHED PRINTOUT EXPLAINS THE ERRORS DETECTED.

PLEASE BE SURE TO FORWARD THIS INFORMATION TO THE APPLICANTS BY EITHER:

1) INCLUDING A COPY OF THIS PRINTOUT IN YOUR NEXT COMMUNICATION TO THE APPLICANTS ALONG WITH A NOTICE TO COMPLY or,

2) CALLING APPLICANTS AND FAXING THEM A COPY OF THE PRINTOUT WITH A NOTICE TO COMPLY

THIS WILL INSURE THAT THE NEXT SUBMISSION RECEIVED FROM THEM WILL BE ERROR FREE.

IF YOU HAVE ANY FURTHER QUESTIONS, PLEASE CALL:

ARTI SHAH 703-308-4212

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION *US/08/822,963*

DATE: 05/05/98
TIME: 14:45:52

INPUT SET: S25541.raw

This Raw Listing contains the General Information Section and those Sequences containing ERRORS.

Does Not Comply
Corrected Diskette Needed

Suggestion: Study Sequence Rules for proper format. ?

```
      1                                SEQUENCE LISTING
      2
      3  (1)    General Information
      4
      5  (i)APPLICANTS:DAKAI LIU
      6  RABBANI, ELAZAR
      7
      8  (ii)TITLE OF INVENTION:VECTORS AND VIRAL VECTORS, AND PACKAGING CELL LINES FOR PRO
      9
-->  10  (iii)NUMBER OF SEQUENCES: __16___
     11
     12  (iv)CORRESPONDENCE ADDRESS:
     13  (A)ADDRESSEE:ENZO THERAPEUTICS, INC.
     14  (B)STREET:C/O ENZO BIOCHEM, INC.
     15  527 MADISON AVENUE, 9TH FLOOR
     16  (C)CITY:NEW YORK
     17  (D)STATE:NY
     18  (E)COUNTRY:USA
     19  (F)ZIP:10022
     20
     21  (v)COMPUTER READABLE FORM:
     22  (A)MEDIUM TYPE: 3.5" Micro Floppy Disk.  1.44 KB
     23  STORAGE
     24  (B)COMPUTER:IBM PC/XT/AT, IBM PS/2 OR COMPATIBLES
     25  (C)OPERATING SYSTEM:PC-DOS
     26  (D)SOFTWARE:  MICROSOFT WORD ___ - ASCII TEXT (DOS)
     27
     28  (vi)CURRENT APPLICATION DATA:
     29  (A)APPLICATION NUMBER: US 08/822,963
     30  (B)FILING DATE:21-MARCH-1997
-->  31  (C)CLASSIFICATION:Not Yet Known
     32
     33  (vii)ATTORNEY/AGENT INFORMATION;
     34  (A)NAME:FEDUS, RONALD C.
     35  (B)REGISTRATION NUMBER: 32,567
     36  (C)REFERENCE/DOCKET NUMBER:ENZ-56
     37
     38  (viii)TELECOMUNICATION INFORMATION
     39  (A)TELEPHONE:(212) 583-0100
     40  (B)TELEFAX:(212) 583-0150
     41
-->  42  (ix)SEQUENCE DESCRIPTION:SEQ ID NO:1:
     43
```

"16" are shown - do not place dashes before or after numeric total; just use 16

ALL text must be visible on page.

delete - does not belong here

Please telephone Arti Shah, 703-308-4212, if you have questions

ERRORED SEQUENCES FOLLOW:

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:9 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:1:

TATCACCGC
ATAGTGGCG

Per 1.822 (l) 6 sequence lines, cumulative base total goes in right 9 ← insert margin of each line.

Invalid - Per 1.822 (j) of Sequence Rules, a nucleotide sequence shall be presented only by a single strand, in the 5' to 3' direction, from left to right

---

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:9 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:2:

ACAAGAAAA
TGTTCTTTT

total ✓

same error throughout listing

---

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:10 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:3:

GTACTAGTTA
CATGATCAAT

✓

---

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:8 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:4:

AGACGTCT
TCTGCAGA

✓

---

(2) INFORMATION FOR SEQ ID NO:5:

```
      93  (i) SEQUENCE CHARACTERISTICS:
-->   94  (A) LENGTH:24 base pairs
      95  (B) TYPE:nucleic acid
      96  (C) STRANDEDNESS:double
      97  (D) TOPOLOGY:linear
      98  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:5:
      99
     100  TGGAATTGTGAGCGGATAACAATT
     101  ACCTTAACACTCGCCTATTGTTAA
     102
```

insert total
1

also,
invalid - Per 1.822 (f) if sequence rules, non-coding bases are divided into groups of 10 bases.

```
     103  (2) INFORMATION FOR SEQ ID NO:6:
     104  (i) SEQUENCE CHARACTERISTICS:
-->  105  (A) LENGTH:4 base pairs
     106  (B) TYPE:nucleic acid
     107  (C) STRANDEDNESS:double
     108  (D) TOPOLOGY:linear
     109  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:6:
     110
     111  TAAT
     112  ATTA
     113
```

```
     114  (2) INFORMATION FOR SEQ ID NO:7:
     115  (i) SEQUENCE CHARACTERISTICS:
-->  116  (A) LENGTH:9 base pairs
     117  (B) TYPE:nucleic acid
     118  (C) STRANDEDNESS:double
     119  (D) TOPOLOGY:linear
     120  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:7:
     121
     122  CATGTAATT
     123  GTACATTAA
     124
```

```
     125  (2) INFORMATION FOR SEQ ID NO:8:
     126  (i) SEQUENCE CHARACTERISTICS:
-->  127  (A) LENGTH:13 base pairs
     128  (B) TYPE:nucleic acid
     129  (C) STRANDEDNESS:double
     130  (D) TOPOLOGY:linear
     131  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:8:
     132
     133  AAAAGTGTGACAT
     134  TTTTCACACTGTA
     135
```

```
     136  (2) INFORMATION FOR SEQ ID NO:9:
     137  (i) SEQUENCE CHARACTERISTICS:
-->  138  (A) LENGTH:11 base pairs
     139  (B) TYPE:nucleic acid
     140  (C) STRANDEDNESS:double
     141  (D) TOPOLOGY:linear
```

(ix) SEQUENCE DESCRIPTION:SEQ ID NO:9:

CCGGAGGACAG
GGCCTCCTGTC

---

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:12 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:10:

ACCGACGTCGGT
TGGCTGCAGCCA

---

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:6 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:11:

ATGATC
TACTAG

---

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:9 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:12:

GCGTGGGCG
CGCACCCGC

---

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
--> **(A) LENGTH:9 base pairs**
(B) TYPE:nucleic acid
(C) STRANDEDNESS:double
(D) TOPOLOGY:linear
(ix) SEQUENCE DESCRIPTION:SEQ ID NO:13:

CAGAACATC
GTCTTGTAG

```
      191  (2) INFORMATION FOR SEQ ID NO:14:
      192  (i) SEQUENCE CHARACTERISTICS:
-->   193  (A) LENGTH:8 base pairs
      194  (B) TYPE:nucleic acid
      195  (C) STRANDEDNESS:double
      196  (D) TOPOLOGY:linear
      197  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:14:
      198
      199  TATATAAA
      200  ATATATTT
      201
```

```
      202  (2) INFORMATION FOR SEQ ID NO:15:
      203  (i) SEQUENCE CHARACTERISTICS:
-->   204  (A) LENGTH:309 base pairs
      205  (B) TYPE:nucleic acid
      206  (C) STRANDEDNESS:single
      207  (D) TOPOLOGY:linear
      208  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:15:
      209
-->   210  GAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGC 1
      211
      212  AGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGG 51
      213
      214  GCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCYCAGGGCCAA 101
      215
      216  GAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAAC 151
      217
      218  CATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTT 201
      219
      220  ATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTG 251
      221
-->   222  CTCCCCGAGCTCAATAAAA 301
      223
```

```
      224  (2) INFORMATION FOR SEQ ID NO:16:
      225  (i) SEQUENCE CHARACTERISTICS:
-->   226  (A) LENGTH:309 base pairs
      227  (B) TYPE:nucleic acid
      228  (C) STRANDEDNESS:single
      229  (D) TOPOLOGY:linear
      230  (ix) SEQUENCE DESCRIPTION:SEQ ID NO:16:
      231
-->   232  ACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATC 1
      233
      234  CGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATC 51
      235
      236  TGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTC 101
      237
      238  AAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACTTTCTAGAGAAC 151
      239
      240  CATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTT 201
```

See next page

241
242 ATTTGAACTAACCGGTCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTG251 incorrect
243
244 CTCCCCGAGCTCAGCTGCG
245 ← insert total
246 (cumulative)
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
--> 264 LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LISTING.10.1.97
--> 265 SEQ ID NO: 1, PAGE 6
266
267 ENZ-56
268
269 ENZ-56
270
271
272

delete

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION *US/08/822,963*

DATE: 05/05/98
TIME: 14:45:59

INPUT SET: S25541.raw

Line	Error	Original Text
10	Number of Sequences (0) Doesn't Equal Actual Count (16)	(iii)NUMBER OF SEQUENCES: __16___
31	Wrong Classification	(C)CLASSIFICATION:Not Yet Known
42	Unknown or Misplaced Identifier	(ix)SEQUENCE DESCRIPTION:SEQ ID NO:1:
46	Entered (9) and Calc. Seq. Length (0) differ	(A)LENGTH:9 base pairs
61	Entered (9) and Calc. Seq. Length (0) differ	(A)LENGTH:9 base pairs
72	Entered (10) and Calc. Seq. Length (0) differ	(A)LENGTH:10 base pairs
83	Entered (8) and Calc. Seq. Length (0) differ	(A)LENGTH:8 base pairs
94	Entered (24) and Calc. Seq. Length (0) differ	(A)LENGTH:24 base pairs
105	Entered (4) and Calc. Seq. Length (0) differ	(A)LENGTH:4 base pairs
116	Entered (9) and Calc. Seq. Length (0) differ	(A)LENGTH:9 base pairs
127	Entered (13) and Calc. Seq. Length (0) differ	(A)LENGTH:13 base pairs
138	Entered (11) and Calc. Seq. Length (0) differ	(A)LENGTH:11 base pairs
149	Entered (12) and Calc. Seq. Length (0) differ	(A)LENGTH:12 base pairs
160	Entered (6) and Calc. Seq. Length (0) differ	(A)LENGTH:6 base pairs
171	Entered (9) and Calc. Seq. Length (0) differ	(A)LENGTH:9 base pairs
182	Entered (9) and Calc. Seq. Length (0) differ	(A)LENGTH:9 base pairs
193	Entered (8) and Calc. Seq. Length (0) differ	(A)LENGTH:8 base pairs
204	Entered (309) and Calc. Seq. Length (319) differ	(A)LENGTH:309 base pairs
210	# of Sequences for line conflicts w/ running total	GAACAGATGGAACAGCTGAATATGGGCCAAACAGG
222	# of Sequences for line conflicts w/ running total	CTCCCCGAGCTCAATAAAA301
226	Entered (309) and Calc. Seq. Length (326) differ	(A)LENGTH:309 base pairs
232	# of Sequences for line conflicts w/ running total	ACGCTTGATCCGGCTACCTGCCCATTCGACCACCAA
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
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264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
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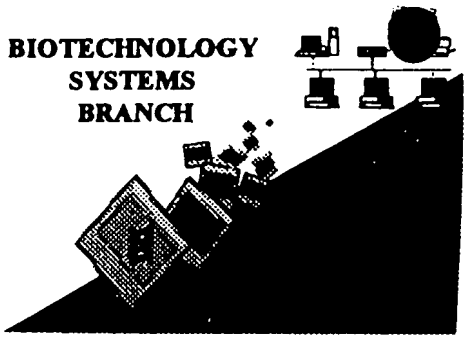
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION *US/08/822,963*

DATE: 05/05/98
TIME: 14:46:00

INPUT SET: S25541.raw

Line	Error	Original Text
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	Wrong Nucleic Acid Designator	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
264	# of Sequences for line conflicts w/ running total	LC/WORD/USPROSECUTION/ENZ56/SEQUENCE LIST
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	Wrong Nucleic Acid Designator	SEQ ID NO: 1, PAGE 6
265	# of Sequences for line conflicts w/ running total	SEQ ID NO: 1, PAGE 6



Notice of Availability of Checker Program

Applicant Aid for Biotechnology Computer Readable Form (CRF)
Sequence Listing Submissions

The Patent and Trademark Office (PTO) has developed a computer program, called Checker, that will aid applicants in identifying and correcting errors prior to making submissions for compliance with the Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures (Sequence Rules: 37CRF 1.821 through 1.825).
Final rules were published in the *Federal Register* (55 FR18230) on May 1, 1990, and in the PTO *Official Gazette* (1114 Off.Gaz.PatOffice 29) on May 15, 1990.

Checker is a DOS-based software program that is intended to assist users in determining whether errors may be present in the sequence listings, and is not intended to guarantee that the submission is error-free.

The most current version of the software is available via computer downloading, details are below. Copies on diskette are also available. Updated software versions will not be automatically mailed out; any updates will be announced in the PTO *Official Gazette*.

The software can be accessed/requested from the following locations:

1) Dial-up access through the Internet. Location is ftp://ftp.uspto.gov
   The software is in current directory: pub/checker/
   Download all the files. Cost: Free-of-charge

3) For diskette copies, mail to: U.S.P.T.O., OEIP, CRYSTAL PARK 3, SUITE 441
   WASHINGTON DC 20231

COST FOR DISKETTE IS $ 25.00
METHOD OF PAYMENT:
Check payable to Commissioner of Patents and Trademarks
VISA/ Mastercard/ Charge- Charges can be faxed to 703-306-2737
PTO Deposit Account

For Further Information, Contact: ***Arti Shah at 703-308-4212***

Application No. 08/822963 File Copy

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821 - 1.825 for the following reason(s):

☑ 1. This application clearly fails to comply with the requirements of 37 CFR 1.821 - 1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.

☐ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c).

☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e).

☑ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached copy of the marked-up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 CFR 1.821(e).

☐ 7.

Other: _____

Applicant must provide:

☑ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing"

☑ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification

☑ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d)

For questions regarding compliance with these requirements, please contact:

For Rules Interpretation, call (703) 308-1123
For CRF submission help, call (703) 308-4212
For PatentIn software help, call (703) 557-0400

Please return a copy of this notice with your response.

08/822,963

Attachment to Paper # 8

```
* * * * * * * * * * * * * * * * * * * * * * * * * * * * *
FILE 'USPAT' ENTERED AT 14:20:28 ON 03 JUN 1998

  * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
  *              W E L C O M E   T O   T H E              *
  *         U. S.   P A T E N T   T E X T   F I L E       *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

=> s snrna

L1          36 SNRNA

=> s l1 and vector?

         66390 VECTOR?
L2          33 L1 AND VECTOR?

=> s l2 and viral

         14281 VIRAL
L3          31 L2 AND VIRAL

=> s l3 and promoter?(5A)snrna

         29977 PROMOTER?
            36 SNRNA
             7 PROMOTER?(5A)SNRNA
L4           6 L3 AND PROMOTER?(5A)SNRNA

=> d l4,1-6,cit,ab
```

1) 5,750,390, May 12, 1998, Method and reagent for treatment of diseases caused by expression of the bcl-2 gene; James D. Thompson, et al., 435/195, 320.1, 325, 336; 536/23.2, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,750,390 [IMAGE AVAILABLE] L4: 1 of 6

ABSTRACT:
An enzymatic RNA molecule which cleaves bcl.2 mRNA associated with development or maintenance of follicular lymphoma.

2. 5,728,521, Mar. 17, 1998, Targeted cleavage of RNA using eukaryotic ribonuclease P and external guide sequence; Yan Yuan, et al., 435/6, 91.2 [IMAGE AVAILABLE]

US PAT NO: 5,728,521 [IMAGE AVAILABLE] L4: 2 of 6

ABSTRACT:
It has been discovered that any RNA can be targeted for cleavage by RNAase P from eukaryotic cells, for example, human RNAase P, using a suitably designed oligoribonucleotide ("external guide sequence", or EGS) to form a hybrid with the target RNA, thereby creating a substrate for cleavage by RNAase P in vitro. The EGS hydrogen bonds to the targeted RNA to form a partial tRNA like structure including the aminoacyl acceptor stem, the T stem and loop, and part of the D stem. The most efficient EGS with human RNAase P is the EGS in which the anticodon stem and loop was deleted. Modifications can also be made within the T-loop. Methods are also disclosed to randomly select and to express a suitable EGS in vivo

to make a selected RNA a target for cleavage by the host cell RNAase P, thus preventing expression of the function of the target RNA. The methods and compositions should be useful to prevent the expression of disease-causing genes in vivo.

3. 5,624,824, Apr. 29, 1997, Targeted cleavage of RNA using eukaryotic ribonuclease P and external guide sequence; Yan Yuan, et al., 435/91.2; 514/44; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,624,824 [IMAGE AVAILABLE] L4: 3 of 6

ABSTRACT:
It has been discovered that any RNA can be targeted for cleavage by RNAase P from eukaryotic cells, for example, human RNAase P, using a suitably designed oligoribonucleotide ("external guide sequence", or EGS) to form a hybrid with the target RNA, thereby creating a substrate for cleavage by RNAase P in vitro. The EGS hydrogen bonds to the targeted RNA to form a partial tRNA like structure including the aminoacyl acceptor stem, the T stem and loop, and part of the D stem. The most efficient EGS with human RNAase P is the EGS in which the anticodon stem and loop was deleted. Modifications can also be made within the T-loop. Methods are also disclosed to randomly select and to express a suitable EGS in vivo to make a selected RNA a target for cleavage by the host cell RNAase P, thus preventing expression of the function of the target RNA. The methods and compositions should be useful to prevent the expression of disease-causing genes in vivo.

4. 5,624,803, Apr. 29, 1997, In vivo oligonucleotide generator, and methods of testing the binding affinity of triplex forming oligonucleotides derived therefrom; Sarah B. Noonberg, et al., 435/6, 91.1, 320.1; 536/24.1 [IMAGE AVAILABLE]

US PAT NO: 5,624,803 [IMAGE AVAILABLE] L4: 4 of 6

ABSTRACT:
The present invention encompasses improved methods and materials for the delivering of antisense, triplex, and/or ribozyme oligonucleotides intracellularly, and RNA polymerase III-based constructs termed "oligonucleotide generators" to accomplish the delivery of oligonucleotides. Also encompassed by the present invention are methods for screening oligonucleotide sequences that are candidates for triplex formation.

5. 5,610,052, Mar. 11, 1997, Enzymatic RNA with activity to ras; James D. Thompson, et al., 435/366, 363; 536/23.2, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,610,052 [IMAGE AVAILABLE] L4: 5 of 6

ABSTRACT:
An enzymatic RNA molecule which cleaves mRNA associated with development or maintenance of colon carcinoma.

6. 5,599,704, Feb. 4, 1997, ErbB2/neu targeted ribozymes; James D. Thompson, et al., 435/325, 6, 91.31, 172.3, 320.1, 366; 514/44; 536/23.1, 23.2, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,599,704 [IMAGE AVAILABLE] L4: 6 of 6

ABSTRACT:
An enzymatic RNA molecule which cleaves mRNA associated with development or maintenance of breast cancer.

=> d kwic,1

SUMMARY:

BSUM(19)

In a third related aspect, the invention features an expression **vector** which includes nucleic acid encoding an enzymatic RNA molecule described above, located in the **vector**, e.g., in a manner which allows expression of that enzymatic RNA molecule within a mammalian cell.

SUMMARY:

BSUM(22)

The . . . is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression **vectors** is primarily feasible using only ex vivo treatments. This limits the utility of this approach. In this invention, an alternative. . .

DETDESC:

DETD(4)

FIG. . . . of a hammerhead motif ribozyme showing stems I, II and III (marked (I), (II) and (III) respectively) interacting with a **viral** target region. The 5' and 3' ends of both ribozyme and target are shown. Dashes indicate base-paired nucleotides.

DETDESC:

DETD(28)

Expression **Vector** While synthetic ribozymes are preferred in this invention, those produced by expression **vectors** can also be used. (See McSwiggen, U.S. Ser. No. 07/884,431, filed May 14, 1992, hereby incorporated by reference herein.) In designing a suitable ribozyme expression **vector** the following factors are important to consider. The final ribozyme must be kept as small as possible to minimize unwanted secondary structure within the ribozyme. A promoter (eg, the human cytomegalovirus immediate early **promoter** or the human U6 **snRNA promoter**) should be chosen to be a relatively strong promoter, and expressible both in vitro and in vivo (e.g., the human. . .

DETDESC:

DETD(29)

A . . . of shorter length can be used to provide good termination and RNA stability. Such hairpins can be inserted within the **vector** sequences to allow standard ribozymes to be placed in an appropriate orientation and expressed with such sequences attached.

DETDESC:

DETD(30)

Poly(A) . . . the 3' end of the ribozyme. These can be provided by either including a poly(A) signal site in the expression **vector** (to signal a cell to add the poly(A) tail in vivo), or by introducing a poly(A) sequence directly into the expression **vector**. In the first approach the signal must be located to prevent unwanted secondary structure formation with other parts of the. . . In the second approach, the poly(A) stretch may reduce in size over time when expressed

in vivo, and thus the [redacted]tor may need to be checked over time. Care must be taken in addit[redacted] of a poly(A) tail which binds [redacted]ly(A). . .

DETDESC:

DETD(69)

Selected . . . of delivery. Routes of administration include intramuscular, aerosol, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal. Expression **vectors** for immunization with ribozymes and/or delivery of ribozymes are also suitable.

DETDESC:

DETD(73)
b. transduction by retroviral **vectors**,

DETDESC:

DETD(78)

At . . . of delivery strategies are useful in the present invention, including: ribozyme modifications, particle carrier drug delivery vehicles, and retroviral expression **vectors**. Unmodified ribozymes, like most small molecules, are taken up by cells, albeit slowly. To enhance cellular uptake, the ribozyme may. . .

CLAIMS:

CLMS(10)

10. A **vector** comprising a nucleic acid encoding the enzymatic RNA molecule of claim 1 operatively linked to a promoter.

CLAIMS:

CLMS(13)

13. A mammalian cell in vitro comprising a **vector** of claim 10.

=> d kwic,2

US PAT NO: 5,728,521 [IMAGE AVAILABLE] L4: 2 of 6

DETDESC:

DETD(14)

The . . . next round of selection. After eight cycles of selection, the resulting pool of double-stranded DNAs is cloned into an appropriate **vector** and sequenced.

DETDESC:

DETD(29)

There . . . human or other eucaryotic equivalents thereof, are introduced into the cell at the desired location for cleavage using a suitable **vector** or other method known to those skilled in the art for introduction and expression of a gene in a cell.

DETDESC:

DETD(33)

The . . . RNAase P in any cell, such as the RNAase P of human cells, can be directed to destroy specific messenger, **viral** or other RNAs by the use of an appropriate EGS RNA.

DETDESC:

DETD(39)

Any . . . product of an oncogene, such as the ras gene product; where the product is not a normal cell component, a **viral** protein, such as one encoded by an essential gene for HIV replication; or a bacterial protein.

DETDESC:

DETD(42)

There . . . two primary mechanisms for delivering the EGS to intracellular RNA that has been targeted for cleavage: diffusion and via a **vector**.

DETDESC:

DETD(43)

As . . . to the infected cells are those in which critical RNA sequences are transcribed in the nucleus. Important examples of the **viral** agents that replicate in the cell nucleus include herpes viruses (including herpes simplex virus, varicella-herpes zoster virus, cytomegalovirus, and Epstein-Barr. . .

DETDESC:

DETD(44)
Vector-mediated delivery of EGS.

DETDESC:

DETD(45)

Preferred **vectors** are **viral vectors** such as the retroviruses which introduce the EGS directly into the nucleus where it is transcribed and released into the. . .

DETDESC:

DETD(46)

Methods for using retroviral **vectors** for gene therapy are described in U.S. Pat. Nos. 4,868,116 and 4,980,286, and PCT application PCT/US89/03794 and PCT/US89/00422, the teachings. . .

DETDESC:

DETD(47)

Defective retroviral **vectors**, which incorporate their own RNA sequence in the form of DNA into the host chromosome, can be engineered to incorporate. . .

DETDESC:

DETD(48)

The . . . the intr[redacted]ction of specific genetic sequ[redacted]es into human cells involves the use of RNA-containing retroviruses which serve as vehicles or **vectors** for high efficiency gene transfer into human cells.

DETDESC:

DETD(49)

RNAase . . . cells and subsequent partial cytoblation. The removed cells can be treated in the laboratory with appropriate EGS compositions (via appropriate **viral vectors**, such as defective **viral vectors**) and then restored to the same individual. The treated cells will develop in the patient into mature hematopoietic cells, including. . .

DETDESC:

DETD(51)

In . . . and possibly to cure, HIV infection, and related diseases of white blood cells which are subject to transformation by retroviral **vectors** carrying EGS. Particular examples of diseases that may be treated using EGS to target RNA for cleavage by RNAase P. . .

DETDESC:

DETD(55)

A preferred composition is a topical composition, for example, for application to a **viral** lesion such as that produced by herpes simplex virus. These will generally contain between 1 .mu.M and 1 mM oligonucleotide/unit. . . locally for release of EGS. Still another preferred composition is a solution or suspension of the EGS in an appropriate **vector** in combination with conventional pharmaceutical vehicles are employed for parenteral compositions, such as an aqueous solution for intravenous injection or. . .

DETDESC:

DETD(68)

The . . . with CAT mRNA. The EGS.sup.CAT (Sequence ID No. 8) fused upstream with a T7 promoter was cloned into a pUC19 **vector**. The EGS.sup.CAT RNA (Sequence ID No. 8) was prepared through in vitro transcription with T7 RNA polymerase. A HindIII-BamHI DNA. . .

DETDESC:

DETD(76)

In . . . the EGS can function in vivo, the EGS.sup.CAT sequence (Sequence ID No. 8) was inserted downstream of a mouse U6 **snRNA** gene **promoter** in a BLUESCRIPT.TM. (Stratagene, La Jolla, Calif.) **vector** forming pEGS.sup.CAT. The EGS.sup.CAT sequence (Sequence ID No. 8) can be transcribed by RNA polymerass III and the transcription can. . .

DETDESC:

DETD(94)

After eight cycles of selection and the resulting pool of double-stranded DNAs were cloned into the BLUESCRIPT.TM. **vector**

(Stratagene, La Jolla, [redacted]lif.) **vector**. Eighteen plasmid DNAs were sequenced using Sequen[redacted] 2.0 (U.S. Biochemicals, Cleve[redacted]d, Ohio).

DETDESC:

DETD(124)

Inhibition of **viral** mRNA expression with human ribonuclease P.

DETDESC:

DETD(125)

Herpes simplex virus was used to demonstrate that EGS can be used to target a **viral** gene in vivo to inhibit **viral** replication. Herpes simplex viruses are DNA-containing viruses that infect cells, induce synthesis of messenger RNAs, which are transcribed to produce enzymes related to DNA synthesis and breakdown: including thymidine kinase, DNA polymerass and a DNA exonuclease, and **viral** DNA and **viral** structural proteins are made and assembled into infectious **viral** particles. The structure and organization of the herpes simplex virus genome is known, for example, as reported by Roizman, Cell,. . .

DETDESC:

DETD(127)

Cell lines and EGS expression **vectors** were then constructed. Five cell lines were constructed by transfecting plasmid DNAs into human 143TK-cells, which can be obtained from. . .

DETDESC:

DETD(131)

Cells . . . then infected with herpes simplex virus using a multiplicity of infection (MOI) of 1 to 1.5 (1 to 1.5 million **viral** particles/1 million cells) in order to resemble a natural infection with virus. RNA was harvested at 4, 8 and 12. . . late genes U.sub.S 10 and U.sub.S 11. The probe is selected to assure the detection of a high level of **viral** mRNA expression over the entire cycle of **viral** infection.

=> d kwic,3

US PAT NO: 5,624,824 [IMAGE AVAILABLE] L4: 3 of 6

DETDESC:

DETD(14)

The . . . next round of selection. After eight cycles of selection, the resulting pool of double-stranded DNAs is cloned into an appropriate **vector** and sequenced.

DETDESC:

DETD(29)

There . . . human or other eucaryotic equivalents thereof, are introduced into the cell at the desired location for cleavage using a suitable **vector** or other method known to those skilled in the art for introduction and expression of a gene in a cell.

DETDESC:

DETD(33)

The . . . RNAase P in any cell, such as the RNAase P of human cells, can be directed to destroy specific messenger, **viral** or other RNAs by the use of an appropriate EGS RNA.

DETDESC:

DETD(39)

Any . . . product of an oncogene, such as the ras gene product; where the product is not a normal cell component, a **viral** protein, such as one encoded by an essential gene for HIV replication; or a bacterial protein.

DETDESC:

DETD(42)

There . . . two primary mechanisms for delivering the EGS to intracellular RNA that has been targeted for cleavage: diffusion and via a **vector**.

DETDESC:

DETD(43)

As . . . to the infected cells are those in which critical RNA sequences are transcribed in the nucleus. Important examples of the **viral** agents that replicate in the cell nucleus include herpesviruses (including herpes simplex virus, varicella-herpes zoster virus, cytomegalovirus, and Epstein-Barr virus),. . .

DETDESC:

DETD(44)
Vector-mediated delivery of EGS.

DETDESC:

DETD(45)

Preferred **vectors** are **viral vectors** such as the retroviruses which introduce the EGS directly into the nucleus where it is transcribed and released into the. . .

DETDESC:

DETD(46)

Methods for using retroviral **vectors** for gene therapy are described in U.S. Pat. Nos. 4,868,116 and 4,980,286, and PCT application PCT/US89/03794 and PCT/US89/00422, the teachings. . .

DETDESC:

DETD(47)

Defective retroviral **vectors**, which incorporate their own RNA sequence in the form of DNA into the host chromosome, can be engineered to incorporate. . .

DETDESC:

DETD(48)

The . . . the introduction of specific genetic sequences into human cells involves the use of RNA-containing retroviruses which serve as vehicles or **vectors** for high efficiency gene transfer into human cells.

DETDESC:

DETD(49)

RNAase . . . cells and subsequent partial cytoblation. The removed cells can be treated in the laboratory with appropriate EGS compositions (via appropriate **viral vectors**, such as defective **viral vectors**) and then restored to the same individual. The treated cells will develop in the patient into mature hematopoietic cells, including. . .

DETDESC:

DETD(51)

In . . . and possibly to cure, HIV infection, and related diseases of white blood cells which are subject to transformation by retroviral **vectors** carrying EGS. Particular examples of diseases that may be treated using EGS to target RNA for cleavage by RNAase P. . .

DETDESC:

DETD(55)

A preferred composition is a topical composition, for example, for application to a **viral** lesion such as that produced by herpes simplex virus. These will generally contain between 1 .mu.M and 1 mM oligonucleotide/unit. . . locally for release of EGS. Still another preferred composition is a solution or suspension of the EGS in an appropriate **vector** in combination with conventional pharmaceutical vehicles are employed for parenteral compositions, such as an aqueous solution for intravenous injection or. . .

DETDESC:

DETD(68)

The . . . changed to make base-pairs with CAT mRNA. The EGS.sup.CAT fused upstream with a T7 promotèr was cloned into a pUC19 **vector**. The EGS.sup.CAT RNA was prepared through in vitro transcription with T7 RNA polymerase. A HindIII-BamHI DNA fragment of the CAT. . .

DETDESC:

DETD(76)

In . . . the EGS can function in vivo, the EGS.sup.CAT Sequence ID No. 8 sequence was inserted downstream of a mouse U6 **snRNA** gene **promoter** in a BLUESCRIPT.sup.TM (Stratagene, La Jolla, Calif.) **vector** forming pEGS.sup.CAT. The EGS.sup.CAT Sequence ID No. 8 sequence can be transcribed by RNA polymerase III and the transcription can. . .

DETDESC:

DETD(94)

After eight cycles of [redacted]lection and the resulting pool [redacted]f double-stranded DNAs w[redacted] cloned into the BLUESCRIPT.su[redacted]M **vector** (Stratagene, La Jolla, Calif.) **vector**. Eighteen plasmid DNAs were sequenced using Sequenase 2.0 (U.S. Biochemicals, Cleveland, Ohio).

DETDESC:

DETD(125)

Herpes simplex virus was used to demonstrate that EGS can be used to target a **viral** gene in vivo to inhibit **viral** replication. Herpes simplex viruses are DNA-containing viruses that infect cells, induce synthesis of messenger RNAs, which are transcribed to produce. . . enzymes related to DNA synthesis and breakdown: including thymidine kinase, DNA polymerase and a DNA exonuclease, and vital DNA and **viral** structural proteins are made and assembled into infectious **viral** particles. The structure and organization of the herpes simplex virus genome is known, for example, as reported by Roizman, Cell,. . .

DETDESC:

DETD(127)

Cell lines and EGS expression **vectors** were then constructed. Five cell lines were constructed by transfecting plasmid DNAs into human 143TK-cells, which can be obtained from. . .

DETDESC:

DETD(131)

Cells . . . then infected with herpes simplex virus using a multiplicity of infection (MOI) of 1 to 1.5 (1 to 1.5 million **viral** particles/1 million cells) in order to resemble a natural infection with virus. RNA was harvested at 4, 8 and 12. . . late genes U.sub.s 10 and U.sub.s 11. The probe is selected to assure the detection of a high level of **viral** mRNA expression over the entire cycle of **viral** infection.

CLAIMS:

CLMS(9)

9. . . . RNA selected from the group consisting of RNA complementary to oncogenes, RNA complementary to tumor suppressor genes, RNA complementary to **viral** genes and RNA **viral** genes, and cellular mRNAs which encode proteins selected from the group consisting of enzymes, hormones, cofactors, antibodies, and growth factors.

CLAIMS:

CLMS(11)

11. The composition of claim 1 wherein the external guide sequence is in a **vector** for introducing the external guide sequence into a cell containing the RNA targeted for cleavage.

CLAIMS:

CLMS(12)

12. The composition of claim 11 wherein the **vector** is a retroviral **vector**.

CLAIMS:

CLMS(15)

15. . . . RNA selected from the group consisting of RNA complementary to oncogenes. RNA complementary to tumor suppressor genes, RNA complementary to **viral** genes and RNA **viral** genes, and cellular mRNAs which encode proteins selected from the group consisting of enzymes, hormones, cofactors, antibodies, and growth factors.

CLAIMS:

CLMS(16)

16. The method of claim 13 further comprising providing the external guide sequence in a **vector** for introducing the external guide sequence into a cell containing the RNA targeted for cleavage.

=> d fro,1

US PAT NO: 5,750,390 [IMAGE AVAILABLE] L4: 1 of 6
DATE ISSUED: May 12, 1998
TITLE: Method and reagent for treatment of diseases caused by expression of the bcl-2 gene
INVENTOR: James D. Thompson, Boulder, CO
Kenneth G. Draper, Boulder, CO
ASSIGNEE: Ribozyme Pharmaceuticals, Inc., Boulder, CO (U.S. corp.)
APPL-NO: 07/936,421
DATE FILED: Aug. 26, 1992
INT-CL: [6] C12N 15/10
US-CL-ISSUED: 435/195, 320.1, 325, 336; 536/23.2, 24.5
US-CL-CURRENT: 435/195, 320.1, 325, 336; 536/23.2, 24.5
SEARCH-FLD: 514/44; 435/320.1, 199, 240.1, 172.3; 935/44, 46; 536/23.2
REF-CITED:

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ART-UNIT: 189
PRIM-EXMR: Deborah Crouch
LEGAL-REP: Lyon & Lyon LLP

ABSTRACT:
An enzymatic RNA molecule which cleaves bcl.2 mRNA associated with development or maintenance of follicular lymphoma.
13 Claims, 1 Drawing Figures

20. 5,324,643, Jun. 28, 1994, Method of conferring resistance to retroviral infection; Wilson Greatbatch, et al., 435/91.32, 91.1, 91.3, 172.3, 372; 536/23.1; 935/3, 6, 34, 70 [IMAGE AVAILABLE]

21. 5,166,057, Nov. 24, 1992, Recombinant negative strand RNA virus expression-systems; Peter Palese, et al., 435/69.1, 172.3, 194, 235.1, 320.1; 935/32, 34, 57 [IMAGE AVAILABLE]

=> d kwic,21

US PAT NO: 5,166,057 [IMAGE AVAILABLE] L6: 21 of 21

SUMMARY:

BSUM(82)

Animal . . . case of brome mosaic virus (BMV), a positive strand RNA plant virus, SP6 transcripts have been used to identify the **promoter** as a 134 nt **tRNA**-like 3' terminus (Dreher, and Hall, 1988, J. Mol. Biol. 201: 31-40). Polymerase recognition and synthesis were shown to be dependent. . .

SUMMARY:

BSUM(89)

As . . . RNA dependent RNA-polymerase and allow for complementation. Additionally, a non-virus dependent replication system for influenza virus is also described. Vaccinia **vectors** expressing influenza virus polypeptides were used as the source of proteins which were able to replicate and transcribe synthetically derived. . .

DRAWING DESC:

DRWD(20)

FIG. . . . were infected with mixtures of recombinant vaccinia viruses (Smith et al., 1986) at an M.O.I. of approximately 10 for each **vector**. After 1.5 hours, synthetic IVACAT-1 RNP was transfected into the virus-infected cells as described (Lutjyes et al., 1989). Cells were. . . no helper virus infection; 3-RNP transfection, no helper virus; 4-RNP transfection, influenza virus as helper; Lanes 5-11-RNP transfection, vaccinia virus **vectors** as helper viruses express the indicated influenza virus proteins.

DRAWING DESC:

DRWD(21)

FIG. 20A-C. Test of various cell lines. A) Cells were infected with vaccinia **vectors** expressing the PB2, PB1 and PA proteins (Lanes 1,3,5,7) or the PB2, PB1, PA and NP proteins (Lanes 2,4,6,8), transfected. . .

DETDESC:

DETD(44)

Alternatively, . . . virus in accordance with the invention. To this end, the influenza virus polymerase proteins may be expressed in any expression **vector**/host cell system, including but not limited to **viral** expression **vectors** (e.g., vaccinia virus, adenovirus, baculovirus, etc.) or cell lines that express the polymerase proteins (e.g., see Krystal et al., 1986,. . . with influenza virus, synthetic RNP's are replicated in cells through the action of influenza virus proteins expressed by recombinant vaccinia **vectors**. In this way we show that the only influenza virus proteins essential for transcription and replication of RNP are the. . .

DETDESC:

DETD(49)

Synthetic . . . in viral RNAs. An additional area of great interest concerns the development of the influenza virus system as a vaccine **vector**.

DETDESC:

DETD(53)

In this regard, the use of genetically engineered influenza virus (**vectors**) for vaccine purposes may require the presence of attenuation characteristics in these strains. Current live virus vaccine candidates for use. . .

DETDESC:

DETD(111)

The . . . insert were cut with EcoRI and BglII. The fragments were purified from acrylamide gel and cloned together into the pPHV **vector** which had been cut with XbaI and BglII. After transformation, white colonies were grown, analysed by endonuclease digestion and selected. . .

DETDESC:

DETD(116)

pIVACAT1 . . . strand). Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer. These 5' and 3' constructs were ligated into pUC19 shuttle **vectors** digested with XbaI and EcoRI, grown up, cut out with EcoRI/BglII (5' region) and XbaI/EcoRI (3' region) and ligated into. . .

DETDESC:

DETD(141)

The . . . genomes. Furthermore, this technology may allow for the construction of infectious chimeric influenza viruses which can be used as efficient **vectors** for gene expression in tissue culture, animals or man.

DETDESC:

DETD(161)

In . . . vaccinia viruses (Smith et al., 1987, Virology, 160: 336-345) and transfected one hour later with the IVACAT-1 RNP. Mixtures of **vectors** expressing the three polymerases (PB2, PB1 and PA) and the

nucleoprotein were use[redacted] Replication and transcription [redacted] the synthetic RNP. . . . recombinan[redacted] accinia viruses. CAT activity [redacted] present in this sample as well as in cells infected with all four vaccinia **vectors** (FIG. 19, lanes 8 and 10). Cells expressing any of the subsets of these four proteins did not produce detectable. . . . is necessary and sufficient for RNP expression and replication in this system. The levels of CAT activity obtained in vaccinia **vector**-infected cells are reproducibly higher than in cells infected with influenza as helper virus. The most probable explanation for this is. . .

DETDESC:

DETD(162)

A . . . using MDBK, Hela, 293 and L cells. In each case, no CAT activity was observed when cells were infected with **vectors** that express only the 3 polymerase proteins but significant CAT activity was obtained if the additional vaccinia-**vector** inducing NP expression was also added.

DETDESC:

DETD(163)

Peviously, . . . Natl. Acad. Sci. USA 83: 2709-2713; Li et al., 1989, Virus Research 12: 97-112). Since replication through recombinant vaccinia virus **vectors** is dependent only on these proteins, it was conceivable that this cell line may be able to amplify and express. . . proteins supported the expression of CAT (FIG. 20B, lane 2). FIG. 20B, lane 3 shows that the minimum mixture of **vectors** needed to induce CAT activity in 3PNP-4 cells are those expressing only the PB1 and PA proteins. Therefore, the steady. . .

DETDESC:

DETD(164)

Since . . . detectable levels of protein in influenza virus infected cells. Accordingly, we used this mutant RNA to examine whether the vaccinia **vector**-expressed influenza proteins induces CAT activity solely through primary transcription of input RNP or can allow for amplification through replication and. . .

DETDESC:

DETD(166)

This recombinant vaccinia **vector** dependent scheme possesses a number of advantages over the use of influenza virus infection to drive the replication of synthetic. . . NP proteins are required for the detection of expressed protein and for replication of RNP. Another advantage of this vaccinia **vector** driven replication scheme is that since the influenza polymerase proteins are expressed from cDNA integrated into the vaccinia virus, the. . .

=> d kwic,20

US PAT NO: 5,324,643 [IMAGE AVAILABLE] L6: 20 of 21

ABSTRACT:
In . . . or more of the infection processes including retroviral replication and assembly into infective viral particles. The method involves introducing a **vector** into a host cell, wherein the **vector** comprises a polynucleotide which directs transcription, within

the host cell, of RNA which is a) complementary or homologous, depending on. . .

SUMMARY:

BSUM(27)

In . . . genetic resistance to retroviral infection upon a host cell is disclosed. The method involves transforming the host cell with a **vector** comprising a polynucleotide directing transcription within the host cell of RNA which (a) is complementary or corresponding to a nucleic. . . replication of the retrovirus when the host cell is infected. The method also involves transforming the host cell with a **vector** comprising a polynucleotide directing transcription within the host cell of RNA which corresponds to sequences which represent a small portion. . .

SUMMARY:

BSUM(28)

Cells upon which resistance to infection is to be conferred, are transformed with a polynucleotide via a **vector**. "Transformation" or "transformed", as those terms are used throughout this specification and the appended claims, is intended to cover any. . .

SUMMARY:

BSUM(33)

The polynucleotide is transformed via a **vector**. Any known **vectors**, including without limitation, **viral vectors**, retroviral **vectors** and plasmids, may be used. Preferably the **vector** is a plasmid. The **vector** can include a promoter and/or a terminator for regulation of the polynucleotide. The final construct (**vector** and polynucleotide) can include one or more promoters and/or terminators including those made part of the polynucleotide as described above. The **vector** can also include a selectable marker for detection and isolation of successfully transformed cells including without limitation antibiotic resistance to. . .

SUMMARY:

BSUM(35)

Nucleic acid constructs, including a polynucleotide as previously described, are also disclosed. The construct can include a **vector** as previously described.

DRAWING DESC:

DRWD(22)

FIG. 20 is a schematic illustration of the RSV **vector** family.

DETDESC:

DETD(4)

The . . . as used herein, refers to one or more nucleotide sequences (polynucleotides or genes) that are inserted into one of the **vectors** chosen from the group of **vectors** including a virus, retrovirus, or plasmid. The polynucleotide sequences described herein are preferably DNA, but could include RNA or a combination thereof, and are integrated into the appropriate **vector** by ligation or other similar techniques.

With reference to Tabl[redacted], there are listed sequences w[redacted]h are illustrative of the po[redacted]ucleotides of the nucleic acid[redacted]nstructs which are inserted into the appropriate **vector**.

DETDESC:

DETD(12)

A **vector** was used to introduce the nucleic acid constructs into a cell. "**Vector**" specifically refers to a flanking nucleic acid sequence which will allow the synthetic polynucleotide to be introduced into a cell and then either inserted into a chromosome or replicated autonomously. Certain **vectors**, e.g., plasmids, may also be used as a means to amplify the constructs of the present invention. The plasmids pRSVneo, pSV2gpt, pSV2neo, pUC19, and pRSVgpt were used as **vectors** for the preferred constructs of the present invention. Plasmids are circular pieces of DNA. They generally have a bacterial origin. . . . a marker gene which confers resistance to ampicillin. Plasmid pRSVgpt has a marker gene which confers resistance to xanthine. Other **vectors**, including without limitation other plasmids, viruses and retroviruses can alternatively be used in practicing the present invention. The plasmids used. . .

DETDESC:

DETD(14)

Recombinant . . . signifies plasmid, "GB" signifies the plasmid was constructed by Greatbatch GenAid, and "neo" signifies the selectable marker in the RSV **vector** family. In constructing recombinant PRSV neo **vectors**, one or more polynucleotides comprising a nucleic acid construct was inserted at either the HindIII restriction site (hereinafter HindIII construct). . . . is designated pGB-neo-B1 (FIG. 18) and in the opposite orientation, pGB-neo-B2 (FIG. 19). Refer to FIG. 20 illustrating the RSV **vector** family. The figures show two illustrations for each restriction site because the nucleic acid constructs were cloned into the plasmids. . .

DETDESC:

DETD(18)

An effective method of delivering the **vector** DNA into the target cell is required if high efficiency transformation is to be achieved. Transformation of potential host cells. . .

DETDESC:

DETD(21)

The . . . regulation of the amount of RNA produced simply by regulating the number of copies of the gene inserted into the **vector**. Further, Pol III promoters tend to be more or less universal in their expression and should function equally well in. . .

DETDESC:

DETD(22)

The . . . upstream region, the transcription initiating region, "Box A" and "Box B" can be taken directly from any highly active, natural **tRNA**. A **tRNA promoter** sequence which has been shown to be particularly strong is the Glu tRNA gene, in mouse. The Glu tRNA gene has the advantage that it is straightforward to use as an active **promoter** and the short **tRNA** sequence which will be transcribed should not have

any effect on the activity of the nucleic acid construct.

DETDESC:

DETD(23)

Another promoter sequence could involve upstream sequences from the **promoter** coming from a natural **tRNA** gene such as Glu tRNA, while transcriptional initiation sequences "Box A", Box B", and all intervening sequences could be supplied. . . This has the advantage of economy and size which will facilitate synthesis and will allow maximum number of polynucleotides per **vector**. In addition, this promoter begins transcription precisely at the 5' end of the nucleic acid construct and ends transcription within. . .

DETDESC:

DETD(27)

To . . . The plasmid designation as previously described indicates that these nucleic acid sequences were cloned into the Hind site of the **vector**. Plasmid #17 was selected to be used in transforming cells, and the resultant transformed cells 5-1 were chosen to be. . . in FIG. 23. The plasmid designation indicates that these nucleic acid sequences were cloned into the Hind site of the **vector**. Plasmid #16 was selected to be used in transforming cells, and the resultant transformed cells, 4 -2, were chosen to. . . illustrated in FIG. 23. Plasmid designation indicates that these nucleic acid sequences were cloned into the Bam site of the **vector**, plasmid #8 was selected to be used in transforming cells, and the resultant transformed cells, 3-1, were chosen to be. . .

DETDESC:

DETD(32)

Another . . . present invention is a synthesized double-stranded DNA sequence operatively linked to the SV40 early promoter sequence contained within a retroviral **vector**. The nucleic acid construct (Anti-PBS Gene Construct, as shown in FIG. 9) is transcribed into an anti-sense RNA molecule which. . .

DETDESC:

DETD(33)

Copies . . . cut to blunt ends using Nae I and Sma I. The nucleic acid constructs were then cloned into the retroviral **vector** LNSX (a gift of Dr. D. Miller, Seattle) which had been previously cut with Stu I. The inserts have been. . . Transfectants were selected for by neomycin resistance with the presence of G418 in the tissue culture medium, since the retroviral **vector** contains the neo gene.

DETDESC:

DETD(34)

To evaluate the resistance to FeLV of a cell line containing the nucleic acid construct in a retroviral **vector**, confluent monolayers of normal and transfected mink cells were challenged with the EECC strain of FeLV. Inhibition of FeLV replication. . .

DETDESC:

DETD(35)

Although this embodim[redacted] describes transfection of the [redacted]nk lung cells with the retroviral **vector**, feline cat embryo cells (NCE) have been successfully infected with retroviral **vector** which had been generated from a commercially available packaging cell line. Since NCE cells containing the retroviral **vector** with insert are selected for in the presence of G418 in the culture media, these cells could be used as. . .

CLAIMS:

CLMS(1)

We . . .

consisting of retroviral replication, reverse transcription, and translation, said method comprising:
Introduction into said host cell in vitro of a **vector** comprising a polynucleotide which is transcribed to RNA, within said host cell, said RNA is complementary to a nucleic acid. . .

CLAIMS:

CLMS(4)

4. The method of claim 1, wherein said **vector** is selected from the group consisting of a **viral vector**, a retroviral **vector** and a plasmid.

CLAIMS:

CLMS(5)

5. The method of claim 4, wherein said **vector** is a plasmid.

CLAIMS:

CLMS(7)

7. The method of claim 4, wherein said **vector** further comprises a first promoter which controls transcription of said RNA within said host cell.

CLAIMS:

CLMS(8)

8. The method of claim 4, wherein said **vector** further comprises a first terminator which controls termination of transcription of said RNA within said host cell.

CLAIMS:

CLMS(9)

9. The method of claim 4, wherein said **vector** further comprises a marker for selection of transformed cells.

CLAIMS:

CLMS(14)

14. . . . the group consisting of retroviral replication, reverse transcription, and translation, said construct comprising a polynucleotide which when introduced by a **vector** into the host cell

in vitro results in tr[redacted]cription of the polynucleotide [redacted]to RNA complementary to the n[redacted]eic acid sequences. . .

CLAIMS:

CLMS(17)

17. The nucleic acid construct of claim 14, wherein said **vector** is selected from the group consisting of a **viral vector**, a retroviral **vector** and a plasmid.

CLAIMS:

CLMS(18)

18. The nucleic acid construct of claim 17, wherein said **vector** is a plasmid.

CLAIMS:

CLMS(19)

19. The nucleic acid construct of claim 17, wherein said **vector** further comprises a first promoter which controls transcription of said RNA within said host cell, and a first terminator which. . .

CLAIMS:

CLMS(20)

20. The nucleic acid construct of claim 17, wherein said **vector** further comprises a marker for selection of transformed cells.

CLAIMS:

CLMS(25)

25. An RNA molecule, produced from the transcription of a polynucleotide of a **vector** which has been introduced into a host cell in vitro, said RNA molecule (a) confers resistance to retroviral infection upon. . .

CLAIMS:

CLMS(26)

26. The RNA molecule of claim 25, wherein said **vector** further comprises a first promoter which controls transcription of said RNA within said host cell.

CLAIMS:

CLMS(27)

27. The RNA molecule of claim 25, wherein said **vector** further comprises a first terminator which controls termination of transcription of said RNA within said host cell.

CLAIMS:

CLMS(28)

28. The RNA molecule of claim 25, wherein said **vector** further comprises a marker for selection of transformed cells.